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Title:

Is the firing rate of motor units in different vastus medialis regions modulated similarly during isometric contractions?

Abstract:

Introduction: Previous evidence suggests the fibres of different motor units reside within distinct vastus medialis (VM) regions. Whether the activity of these motor units may be modulated differently remains unknown. Here we assess the discharge rate of motor units detected proximo-distally from VM to address this issue.

Methods: Surface electromyograms (EMGs) were recorded proximally and distally from VM while ten healthy subjects performed isometric contractions. Single motor units were decomposed from surface EMGs. The smoothed discharge rates of motor units identified from the same and from different VM regions were then cross-correlated.

Results: During low-level contractions, the discharge rate varied more similarly for distal (cross-correlation peak; interquartile interval: 0.27-0.40) and proximal (0.28-0.52) than for proximo-distal pairs of VM motor units (0.20-0.33; P=0.006).

Discussion: The discharge rates of motor units from different proximo-distal VM regions show less similarity in their variations than those of pairs of units either distally or proximally.

Keywords: motor unit; vastus medialis; surface electromyography; compartmentalization; knee; quadriceps.

Introduction

Architectural differences seem to account for functional differences within the vastus medialis (VM) muscle. Previous studies reported that distal VM fibres are oriented more obliquely to the quadriceps tendon than the proximal fibres[1,2]. In their review, Smith et al.[3] specifically observed the orientation of VM distal and proximal fibres in relation to the quadriceps tendon respectively ranges from 40˚ to 77˚ and from 11˚ to 35˚. Furthermore, *in-vivo*[4,5] and cadaveric[1] studies reported that distal VM fibres attached directly to the medial edge of the patella. Collectively, these findings indicate the activation of fibres in different, proximo-distal VM regions results in force vectors oriented in different directions[6]. Such differential, architectural organisation has led to the consideration that fibres in distinct VM regions may contribute to distinct functions[2,6]. Whether the nervous system may selectively activate fibres in distinct VM regions is an open and potentially relevant issue for the rehabilitation of patients with knee musculoskeletal disorders such as patellofemoral pain syndrome[7,8].

The possibility of controlling distinct VM regions presumes a specific, neuromuscular organisation. First, VM motor units (MUs) must have small territories in relation to the muscle proximo-distal axis. Otherwise, activation of individual motor neurones would result in contraction of fibres spanning a large muscle region, resulting in force vectors with similar directions for different MUs. Investigations using scanning electromyography indicated that in-depth territory of VM motor units is remarkably small, ranging between 2 to 8 mm in healthy subjects[9]. More recently, Gallina and Vieira[2] observed the action potentials of single MUs are represented locally proximo-distally in the VM, suggesting that the territory of some VM motor units is relatively small. In agreement with this view, Lin et al.[6] observed that the patella shifted medially when stimulation pulses were delivered to the distal VM region and proximally when the proximal VM region was stimulated. In addition to MUs with small territories, the

activation of distinct VM regions demands preferential access to these MUs within the nervous system; if all VM motor neurones receive a common input, the activity of different proximo-distal VM fibres would be modulated similarly.

In this study, we therefore investigated whether the activity of MUs represented in surface electromyograms (EMGs) detected from different VM regions are modulated differently. We specifically asked: is the firing rate of MUs identified proximally and distally in the VM modulated equally strongly during isometric, torque-varying contractions? If MUs with territories in different VM regions receive different inputs, then, we expect the firing rate of pairs of MUs identified from the same VM region, either distal or proximal, to be modulated more similarly than that of pairs of units taken from different VM regions. Addressing this question would contribute to advancing our knowledge of the functional organisation of MUs within the VM.

Methods

Participants

Ten healthy, male subjects (range: 24-32 years; 168-182 cm; 70-85 kg) were recruited to participate in the study. Participants did not report any knee injuries at the time of the experiments and all provided written informed consent. The study was conducted in accordance with the latest revision of the Declaration of Helsinki and approved by our University Hospital Ethics Committee (HUCFF/UFRJ – 127/2013).

Experimental protocol

Knee extension torque was measured with participants seated comfortably on a dynamometer chair (Biodex System 4, New York, USA), with their right knee flexed at 80˚ and aligned as coaxially as possible to the dynamometer axis of rotation. Two isometric, maximal voluntary contractions (MVCs) lasting 5 s each were performed, with a rest period of at least 2 min in-between. The peak torque, averaged across the two MVCs, was considered as the maximal knee extension torque. After that, torque-varying, isometric contractions at two force levels were applied. Participants were asked to increase knee torque from rest to a submaximal target level in 5 s, to hold it at that level for 10 s and then to return to rest in 5 s. This trapezoidal profile was repeated four times for each of two contraction levels, 20% and 40% MVC. The contraction level was randomised and a rest period of at least 5 min was provided in-between. Visual feedback of knee extension torque was presented on a computer monitor. Data collection started after participants had trained with visual feedback and could successfully follow the trapezoidal profiles; the familiarisation session started at least 3 min after MVCs.

Electrode placement and EMGs recordings

Two adhesive arrays of eight, silver-bar electrodes each (10 mm inter-electrode distance; Spes Medica, Battipaglia, Italy) were used to sample surface EMGs. The reference electrode was placed over the patella and conductive paste (TEN 20 Conductive Paste, Weaver, Aurora, USA) was used to assure electrical contact between electrodes and skin. Prior to positioning the arrays, the skin was shaved and cleaned with abrasive paste. With a dry array of eight silver-bar electrodes (LISiN-Politecnico di Torino, Turin, Italy), EMGs were visually inspected while participants gently, isometrically loaded their knee extensors. The array orientation was then changed until the propagation of action potentials of individual MUs could be clearly observed across electrodes; this orientation was deemed parallel to the average orientation of fibres underneath electrodes[2,10]. This procedure was repeated with the dry array centred roughly at the VM distal and proximal regions, defined through palpation. Adhesive arrays were then centred at these locations. An example of the position of adhesive arrays and the propagation of motor unit action potentials is provided in Figure 1.

Surface EMGs were recorded in monopolar derivation and amplified by a variable factor, ranging from 2,000 to 10,000 (10-900 Hz bandwidth amplifier; CMRR > 100 dB; EMG-USB2, OTBioelettronica, Turin, Italy). EMGs and the torque signal were digitised synchronously at 2048 samples/s using a 12-bit A/D converter, with 5 V dynamic range.

Assessing the variation in motor unit firing rates

Raw surface EMGs were first visually inspected for power line interference and contact problems. Low-quality EMGs were not observed among the 320 (10 subjects x 8 electrodes x 2 VM portions x 2 contraction levels) monopolar signals collected.

Similarities in modulation of MUs detected from different VM regions were assessed through cross-correlation. First, monopolar EMGs were band-pass filtered with a 4th order Butterworth filter (15-350 Hz cut-off frequencies). Filtered EMGs were decomposed into their constituent trains of motor unit action potentials[11,12]. Decomposition was applied separately for each contraction level and array of electrodes, providing the firing instants of MUs recruited for different effort levels and represented in different VM regions. The coefficient of variation of the inter-spike interval was computed for each MU considering periods of constant torque. Additionally, the instantaneous firing rate of individual MUs was computed as the multiplicative inverse of the inter-spike interval, linearly interpolated at 50 Hz, smoothed with a low-pass Butterworth filter (4th order, 3 Hz cut-off frequency[12]) and demeaned. For each participant, the resulting, smoothed firing rate profiles were cross-correlated for pairs of MUs: i) obtained from the same array, either proximal or distal; ii) identified from different arrays. The normalised, cross-correlation functions were calculated considering the longest period within which all MUs were active, separately for each of the four, consecutive trapezoidal contractions. The peak of the cross-correlation function, taken for lags from -100 to 100 ms[13] and averaged across the four consecutive contractions, was considered a measure of how similarly the firing rate of pairs of MUs varied for each individual and contraction level.

Only MUs recruited at torque levels lower than 90% of the target level were considered for analysis. The recruitment threshold of individual units was calculated as the torque value observed at the time of the first discharge, averaged across the four, trapezoidal contractions[14]. Moreover, MUs whose action potentials were clearly represented in both arrays of electrodes or that did not discharge continuously (< 200 discharges; 4 repetitions x 10 s plateau x 5 discharges per second) were also excluded from analysis. Figure 2 shows examples of surface EMGs triggered and averaged at the time of the firing of individual MUs identified proximally

and distally from VM. While each of the two MUs shown in Figure 2A is represented mainly either in the distal or proximal array, potentials of the MUs shown in Figure 2B appear clearly in both arrays.

Statistics

Given the data distribution was not Gaussian (Kolmogorov-Smirnov test; $P < 0.029$) non-parametric analysis was considered for inferential statistics. Wilcoxon rank sum test was applied to compare the recruitment threshold of MUs identified for 20% and 40% MVC. The strength of the relationship between recruitment threshold and the interquartile interval of the MU firing rates was assessed with Pearson correlation analysis. The Kruskal-Wallis test was applied separately for 20% and 40% MVC to test for differences in the peak of the cross-correlation function computed for pairs of MUs in the same and in different VM regions; the Dunn-Sidak test was used for post-hoc comparisons. Statistical analysis was carried out with Matlab (Version 8.5, The MathWorks Inc., Natick, Massachusetts, USA). The level of significance was set at 5%.

Results

A total of 80 MUs were identified for the ten participants. Six MUs were excluded because their action potentials were observed in both arrays; all were identified proximally during 40% MVC. The firing characteristics of all MUs analysed are shown in Table 1 (20% MVC) and Table 2 (40% MVC), separately for each subject and VM region. A median of 4 MUs were analysed per subject and contraction level.

Motor units recruited for the different contraction levels

During the 20% MVC trapezoidal contraction, MUs first discharged when knee torque reached 8.0% (6.2%-11.5%) MVC (median and interquartile interval). Conversely, the first discharge of MUs decomposed during the 40% MVC were observed at a significantly greater torque value (25.6%, 19.0%-29.0% MVC; Figure 3; Wilcoxon test; $P < 0.0005$; $N = 74$ MUs, 40 distal and 34 proximal units). As shown in Figure 3, variations in the MU discharge rates were significantly negatively correlated with recruitment threshold, both for 20% and 40% MVC contractions (Pearson $R < -0.47$ and $P < 0.002$ for both cases). MUs recruited at higher torque levels showed less variation in firing rate.

Cross-correlation function

The firing rate of MUs identified from EMGs detected proximally and distally was modulated differently. Figure 4 shows the smoothed, instantaneous firing rate of four MUs decomposed for a representative participant and the resulting, cross-correlation functions. All four MUs were recruited at torque values well below the target, 20% MVC torque level (Figure 4A). Two of these units were decomposed from EMGs collected distally and their action potentials were clearly represented in the distal array of electrodes, whereas the two MUs decomposed proximally were represented predominantly in EMGs detected proximally (cf. bottom and top

206 traces in Figure 4B). The cross-correlation functions calculated for pairs of MUs decomposed
207 from the same array of electrodes showed a clear, single peak at zero lag (Figure 4C). In
208 contrast, pairs of units decomposed from different arrays either showed a markedly small cross-
209 correlation value around 0 s or did not show a distinct peak.
210
211 Group data revealed the firing patterns of MUs decomposed for either the proximal or distal
212 VM region were more similarly modulated than those of units decomposed proximo-distally.
213 Cross-correlation functions were computed for 116 pairs of MUs; 28 pairs of distal units, 16
214 pairs of proximal units and 73 proximo-distal pairs. Of the distal, proximal and crossed pairs,
215 17, 9 and 39 were respectively obtained for the 20% MVC contraction and, then, there were
216 instances with no or one motor unit being identified from a given VM region. The distribution
217 of the peak of the cross-correlation function is shown in Figure 5 for all MU combinations.
218 Kruskal-Wallis test revealed a significant difference in cross-correlation values between VM
219 regions at 20% MVC. The firing rate variation was significantly more similar for pairs of MUs
220 identified from the same VM region, both distal (interquartile interval: 0.27-0.40) and proximal
221 (0.28-0.52), than for pairs of units in different regions (0.20-0.33; $P = 0.006$). For 40% MVC,
222 no significant differences in cross-correlation peaks were observed for any pairwise
223 comparisons between distal (0.23-0.40), proximal (0.27-0.36) and crossed MUs pairs (0.22-
224 0.33; $P = 0.668$).

Discussion

Our main finding showed that MUs decomposed during 20% MVC from the same array, either distal or proximal, discharged with variations that were significantly more similar than those of units decomposed from different arrays. As discussed below, at least for low-level contractions, these results suggest motor neurones serving predominantly either the distal or proximal VM fibres may receive different synaptic input. The differential activation of distal and proximal VM motor units may therefore contribute to regulating the direction of the knee extension, force vector.

Assessing different populations of vastus medialis motor units

Notwithstanding the validity of the decomposition algorithm[12,15], decomposition results are typically limited to the identification of a relatively small number of MUs[16]. Among the MUs recruited, those with greater amplitude are more likely to be identified for a given, fixed contraction level[17]. Results shown in Figure 3 suggest, indeed, different populations of MUs were identified for 20% and 40% MVC. Moreover, corroborating previous findings[14,18], MUs recruited at progressively greater torque levels discharged at lower rates (Figure 3). Even though the results presented here may not be generalised to all VM MUs, they apply to MUs recruited at torque levels often elicited during daily activities[19].

Is the activity of proximo-distal motor units modulated differently in vastus medialis?

Different methods have been proposed to assess how similarly the activity of different MUs is modulated[20]. In agreement with previous studies[13,21-23], here we assessed similarities in the activity of VM motor units using the cross-correlation function. This association between motor neurone activity and cross-correlation function is a corollary following the well-established notion that variations in the synaptic drive impinging upon a given population of

active motor neurones lead to a concurrent and similar variation in their firing rate[18,24]. Interestingly, our key results show significant differences for 20% MVC in the cross-correlation function evaluated for pairs of units detected from the same and from different VM regions. When considering pairs of units detected from the same VM region, cross-correlation values 0.27-0.52 (interquartile interval) were somewhat similar to those reported in the literature for other muscles (typically within the 0.3-0.6 range[13,21,22,25-27]). When considering crossed-pairs of units (proximal-distal), cross-correlation values were ~30% smaller (0.20-0.33; Figure 5). In view of these arguments, our results are consistent with the possibility that motor neurones serving fibres located in different VM regions do not share the same, synaptic input during isometric, knee extension contractions.

According to the common drive principle, the firing rate of active MUs is modulated in unison[23,28] with the active MUs sharing the same synaptic input. However, it is known that activation of different volumes of individual muscles, in particular of those with broad attachment, contributes to different joint motions (e.g., deltoid[29] and trapezius[30]). Such selective activation of muscles sub-volumes implies that motor neurones serving different sub-volumes receive distinct, net inputs. Following this reasoning, it is possible that different pools of MUs, each elicited for a specific purpose (e.g. to regulate force direction or to endure a fatiguing contraction[13,31,32]), receive different inputs. Results presented here suggest this concept may be extended, at least in VM, to motor neurones serving different, proximo-distal muscle regions.

A final consideration on the regional modulation of MU firing rates concerns the different contraction levels. During 40% MVC, the cross-correlation peak did not differ between pairs of crossed units and of units detected from the same region (Figure 5). Different factors may

have contributed to the lack of statistical significance. First, it is possible that the proximo-distal differentiation of MUs' activity becomes less relevant for torque demands higher than 20% MVC, as a relatively greater proportion of VM motor units is recruited. Second, as discussed below, we cannot exclude the possibility that fibres of some MUs spanned an extensive, proximo-distal VM region. Third, in the EMGs, the interferential activity of different MUs increases markedly with the contraction level[33], hampering the distinction of action potentials of individual MUs. As a consequence, the decomposition of EMGs collected at greater force levels may not work as effectively as it does at lower contraction levels[33].

What are the potential causes and consequences of regional differences in motor unit modulation?

In this study, MU firings observed in different arrays are expected to reflect the discharges of motor neurones innervating fibres localised proximally or distally. Due to the close association between the location of active muscle fibres and their EMGs amplitude distribution, surface potentials are greater when collected above than far from the MU territory[34]. Indeed, when regional activation is elicited through selective, intra-muscular VM stimulation, the peak of the EMGs distribution can be observed near the stimulating electrode and signals recorded from electrodes 60 mm far from the distribution peak are dramatically small[35]. For these reasons, motor unit action potentials identified in either array in this study likely belong to units located predominantly proximally or distally within the VM.

During low-level contractions, MUs in different VM regions may be recruited based on their function. Due to distributed insertion along the patella medial edge[4] and to variations in fibre orientation within the VM[3], distinct VM regions may contribute differentially to the patellar, proximo-medial translation[6]. The nervous system may take advantage of this anatomical

arrangement to preferentially recruit populations of VM fibres producing force in specific directions. In such case, fluctuation in the neural drive would affect MUs serving fibres within relatively small VM regions[2]. Our current findings seem to support this hypothesis (Figure 4 and 5). Given the spectrum of orientations of VM fibres[2], the potentially weak, mechanical linkage between VM fibres[6] and the differential modulation of activity of proximal and distal MUs (Figure 4 and 5), it is therefore possible the nervous system relies on the activation of different MUs to specifically control force direction in the VM. If this is the case, subjects could learn or be trained to selectively activate distinct VM regions (for example, through EMG biofeedback), opening new fronts for the rehabilitation of patients with knee musculoskeletal disorders such as patellofemoral pain syndrome.

Abbreviations

EMGs – surface electromyograms

IZ – innervation zone

MUs – motor units

MVC – maximal voluntary contraction

VM – vastus medialis

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Tables

Table 1: Firing characteristics of motor units analysed for 20% MVC contractions, separately for each subject and vastus medialis region.

Subjects	*Number of MUs*		*Recruitment threshold (%MVC)*		*Mean inter-spike interval (s)*		*Coefficient of variation of inter-spike interval*	
	Prox	Dist	Prox	Dist	Prox	Dist	Prox	Dist
#1	3	2	4.22-6.53	7.88-8.74	0.07-0.11	0.08-0.09	0.39-0.50	0.32-0.38
#2	2	2	2.63-6.19	5.87-11.55	0.09-0.10	0.11-0.14	0.38-0.45	0.39-0.43
#3	2	3	7.00-13.18	11.03-16.89	0.11-0.12	0.12-0.16	0.37-0.44	0.31-0.42
#4	1	0	16.11		0.13		0.36	
#5	2	4	6.99-8.89	5.01-8.41	0.08-0.10	0.08-0.09	0.29-0.38	0.19-0.39
#6	2	2	4.72-8.25	6.13-10.13	0.08-0.13	0.08-0.09	0.37-0.48	0.37-0.41
#7	2	2	13.99-14.74	2.67-6.55	0.11-0.13	0.09-0.10	0.44-0.48	0.45-0.47
#8	1	3	14.44	7.71-16.00	0.09	0.11-0.14	0.35	0.41-0.50
#9	0	1		10.95		0.08		0.43
#10	2	2	6.23-8.87	4.84-11.98	0.07-0.08	0.09-0.12	0.32-0.43	0.43-0.47

MUs: motor units; MVC: maximal voluntary contraction; Prox: proximal array; Dist: distal array.

Table 2: Firing characteristics of motor units analysed for 40% MVC contractions, separately for each subject and vastus medialis region.

Subjects	*Number of MUs*		*Recruitment threshold (%MVC)*		*Mean inter-spike interval (s)*		*Coefficient of variation of inter-spike interval*	
	Prox	Dist	Prox	Dist	Prox	Dist	Prox	Dist
#1	2	2	21.61-27.13	29.79-32.24	0.08-0.09	0.09-0.14	0.36-0.38	0.41-0.49
#2	2	2	20.38-30.14	16.42-18.31	0.10-0.11	0.07-0.09	0.37-0.49	0.32-0.43
#3	1	1	13.02	14.12	0.08	0.08	0.36	0.34
#4	2	3	26.88-33.53	10.90-26.25	0.11-0.11	0.10-0.10	0.40-0.41	0.44-0.48
#5	2	2	20.01-23.84	9.47-25.46	0.10-0.11	0.08-0.10	0.32-0.45	0.37-0.40
#6	2	3	20.06-27.56	29.12-29.92	0.08-0.09	0.09-0.12	0.35-0.38	0.39-0.44
#7	2	1	16.56-29.01	28.59	0.11-0.12	0.09	0.49-0.50	0.44
#8	1	2	18.98	13.13-19.05	0.11	0.08-0.11	0.50	0.36-0.41
#9	1	1	25.75	27.83	0.08	0.11	0.46	0.50
#10	2	2	22.22-31.65	27.82-29.78	0.12-0.13	0.09-0.10	0.46-0.47	0.35-0.39

MUs: motor units; MVC: maximal voluntary contraction; Prox: vastus medialis proximal region; Dist: vastus medialis distal region.

Figure captions

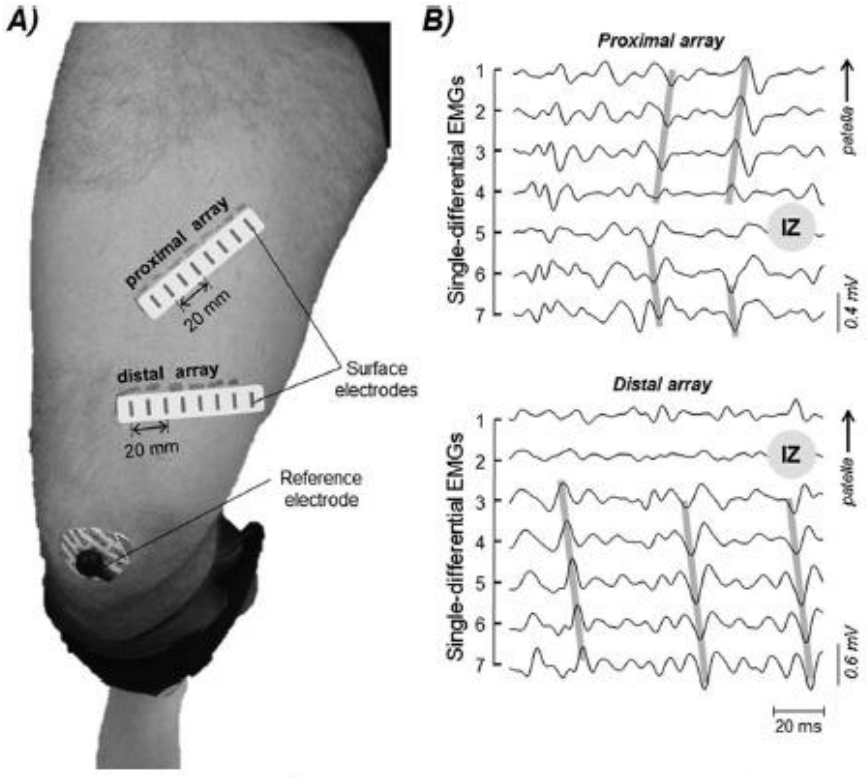

Figure 1: Electrode positioning and raw, differential electromyograms.

A schematic representation of the position of surface electrodes over the vastus medialis (VM) muscle is shown in panel *A*. A short epoch (100 ms) of raw, single-differential EMGs detected by both arrays is shown in panel *B*. Innervation zone (IZ; shaded circles) and propagation (thick, grey lines) of motor unit action potentials are clearly seen in both VM regions, indicating both arrays were aligned roughly parallel to VM proximal and distal fibres.

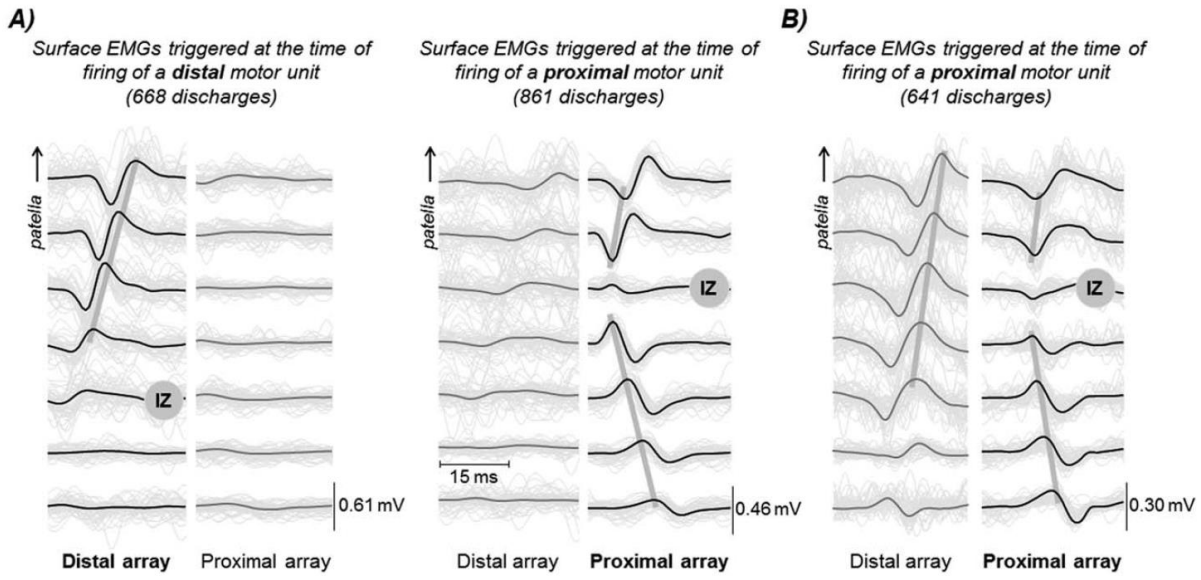

Figure 2: Templates of two motor units (one distal and one proximal).

A, shows action potentials of motor units decomposed from EMGs detected distally (left column; *N* = 668 firings) and proximally (right column; *N* = 861 firings). Black traces correspond to the average of grey traces at the time of the motor unit firings. Note the action potentials of each of the two motor units are more clearly represented in the array from which they were decomposed. As shown in panel *B*, the action potentials of a motor unit decomposed from proximal EMGs, for this same participant, demonstrate similar amplitude in both arrays. Note innervation zones (IZ; shaded circles) and propagation (thick, grey traces) can be observed clearly in the signals.

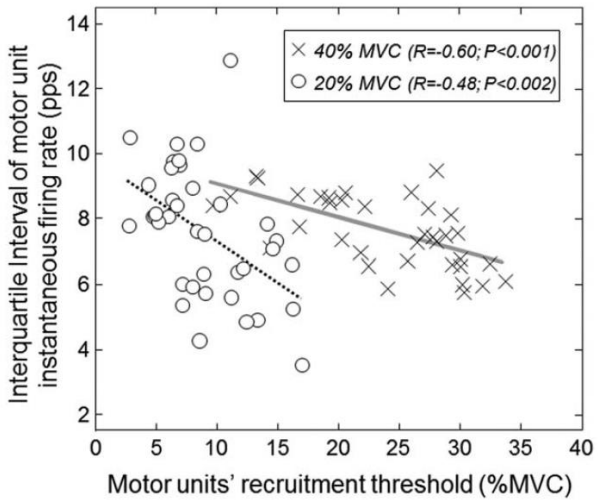

Figure 3: Motor unit firing rates and recruitment threshold.

The scatter plot shows how changes (ordinate; interquartile interval) in the firing rate of motor units varied with the torque value matching the units' first discharge (abscissa; recruitment threshold). Motor units decomposed from surface EMGs detected during 20% MVC (N = 38) are represented with circles whereas crosses denote units decomposed for 40% MVC contractions (N = 36).

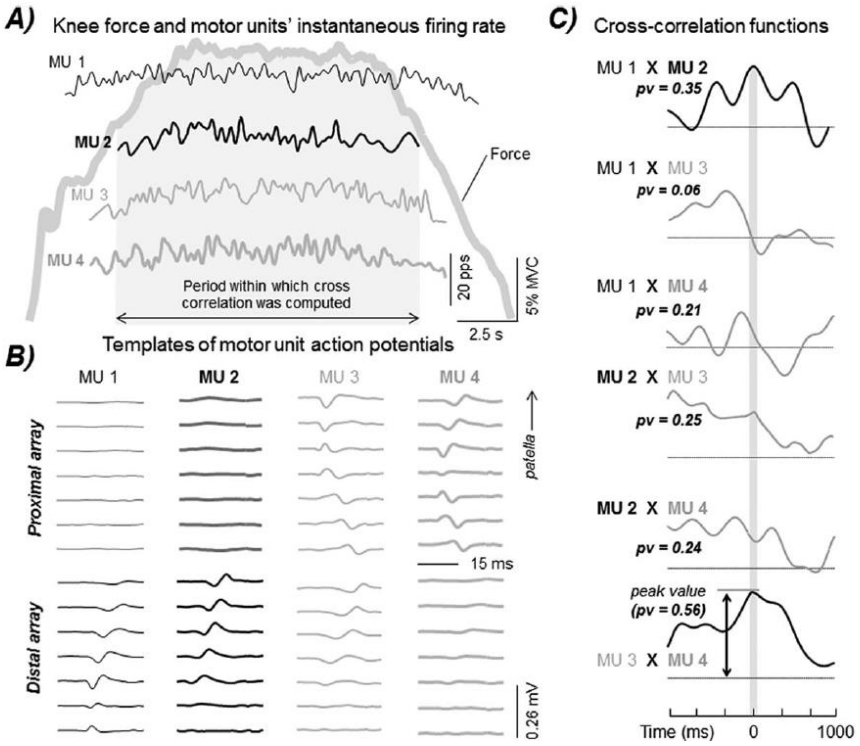

Figure 4: Example of regional modulations in the activity of vastus medialis motor units.

A, shows the knee extension torque and the smoothed, instantaneous firing rate of four decomposed motor units for a single, representative participant during one trapezoidal, torque-varying contraction. The firing rate of units decomposed proximally is represented with black traces while that of distal units is shown with grey traces. The recruitment threshold of MU1, MU2, MU3 and MU4 were respectively 4.8, 12.0, 8.9 and 6.2% MVC. The spike-triggered, average representation of the action potential of each of the four decomposed motor units is shown in panel *B*. Note the action potentials of units decomposed proximally and distally

appear respectively predominantly in the proximal and distal arrays. *C*, shows the cross-correlation functions computed for the six possible combinations of pairs of proximal and distal motor units. The vertical, grey rectangle indicates the lag for which cross-correlation values were considered to compare how similarly the firing rate of pairs of motor units was modulated.

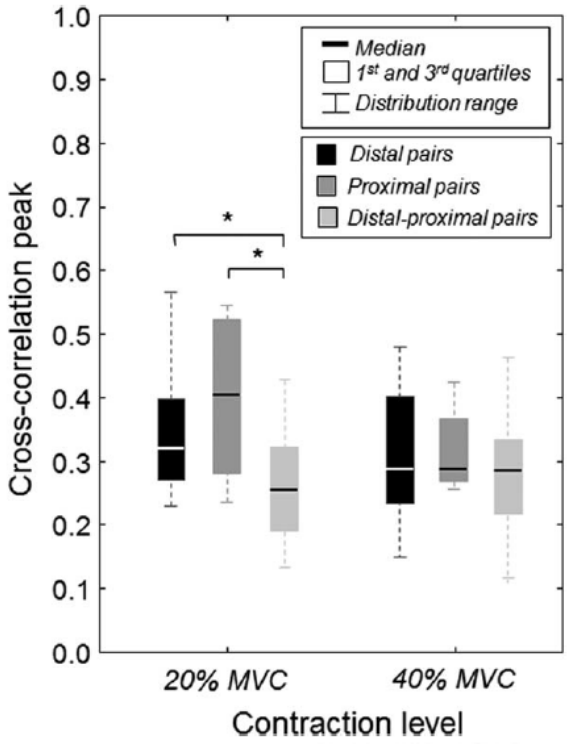

Figure 5: Cross-correlations of vastus medialis motor units.

The distribution of peak values of the cross-correlation functions computed for pairs of distal (black boxes), proximal (dark grey boxes) and proximo-distal (light grey boxes) motor units is shown separately for 20% and 40% MVC contraction levels. Horizontal traces, boxes and whiskers respectively denote the median value, the interquartile interval and the distribution range. Asterisk denotes statistical significance ($P < 0.05$).